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14. ABSTRACT
The study aims include investigating specific mechanisms of adverse effects related to RBC storage age in critically ill patients. We continue to enroll patients in this study. Thus far, we have enrolled 82 patients, from whom samples have been collected, processed, shipped from Canada to San Francisco. We have completed coagulation testing on 90 patients' samples and HLA typing for 54 patients' Day 0 samples so far. Optimization work for the microparticle studies have built on related NIH-funded studies of in vitro properties of microparticles, refining the list of activation markers to be tested. We have completed microparticle testing for 6 patients.

15. SUBJECT TERMS
RBC storage age; microchimerism; critically ill patients; coagulation; microparticles

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Adverse Effects of RBC Storage in Critically Ill Patients

INTRODUCTION

Combat casualties are specifically at risk of adverse effects resulting from the use of RBCs of increased storage age. A large multicenter randomized controlled trial in 30 Canadian centers of 2500 critically ill patients called the Age of Blood Evaluation (ABLE) trial has been funded. In this trial of critically ill patients, which includes patients with traumatic injuries, study groups will be randomized to either RBCs of < 8 days storage time or standard RBC storage time (mean 21 days). The primary outcome of this trial is 90 day mortality. Secondary outcomes include severity of multiple organ dysfunction syndrome, serious thrombotic events and nosocomial infections, and ICU and hospital length of stay. Prospective clinical studies investigating the mechanisms and clinical outcomes associated with increased or decreased RBC storage age in critically ill patients including traumatic injury have not been performed. The ABLE study presents a unique and probably one-time opportunity to investigate mechanisms in the context of clinical outcomes for well-characterized study groups. This study is designed to determine specific mechanisms of adverse effects related to the RBC storage age in transfused critically ill patients enrolled in the ABLE study. This ancillary study will specifically determine if the RBC unit storage time affects patient's Immune function, inflammation, coagulation, microparticle concentrations and microchimerism.

Aims

1. To determine how RBC unit storage time affects coagulation in 100 critically ill patients, how these effects change over time after transfusion and if these parameters correlate with clinical outcomes.
    1a. Quantify levels of Prothrombin Fragments 1+2, soluble Thrombomodulin, Protein C, PAI-1, tissue Plasminogen Activator, Factors V, VII, VIII, D-Dimer, Antithrombin III, soluble Endothelial Protein C Receptor, Xia, INR, PT & PTT using standard testing methods.
    1b. Correlate patterns of measures of coagulation with receipt of blood stored for short vs. long periods.
    1c. Correlate patterns of coagulation with inflammatory markers, immune function, microparticle concentration and clinical outcomes.
2. To determine if RBC unit storage time affects microparticle concentrations in both the RBC unit that is transfused and in 100 critically ill patients who are transfused. We will determine if microparticle concentrations correlate with coagulation, inflammation, altered immune function and clinical outcomes.
    2a. Quantify the concentration of microparticles in the stored blood product from RBC unit segments and patient plasma before and after transfusion using Fluorescent Activated Cell Sorting (FACS) analysis.
    2b. Define the likely cellular source of microparticles in the stored blood product and transfusion recipients using a panel of flow cytometry antibodies to define of the microparticles.
    2c. Define the phenotype of microparticles in the stored blood product and transfusion recipients by staining them with panels of antibodies to activation markers.
    2d. Correlate with systemic markers of coagulation, inflammation and immune dysfunction.

2e. Correlate microparticle concentration and activation marker profile with clinical outcomes.

3. To determine the incidence and magnitude of TA-MC in 200 critically ill patients.

3a. Determine the HLA type of transfusion recipients.

3b. Measure the presence of minor populations of non-self cells based on panels of insertion-deletion polymorphisms and HLA class II allele disparities using highly sensitive real-time Polymerase Chain Reaction (PCR) assays.

3c. Correlate TA-MC results with RBC storage age in addition to immune function and inflammation results from previously funded studies of the same patients enrolled in this study.

BODY

As of December 26th, 2012, we have enrolled a total of 82 patients in this study. Evaluable samples have been collected, processed and shipped from the clinical site to BSRI.

Aim 1: Optimization of the coagulation marker measurements has been completed. We have started performing coagulation testing on patient samples in batches (see Fig. 1 for representative markers). However these results can neither be correlated with patient status of receiving old or new blood nor with clinical outcomes, as that information will only be available once the study is completed. We have completed coagulation testing on 90 patients' samples.

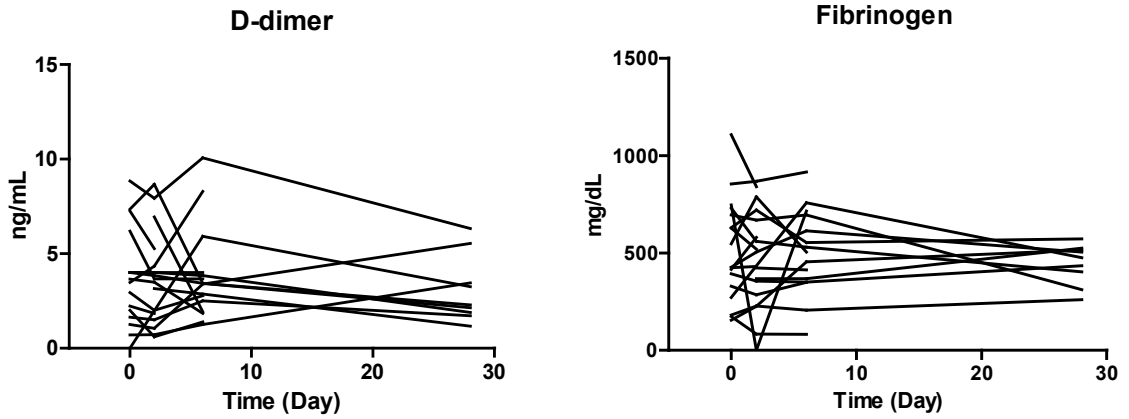

Fig. 1 Evolution of coagulation markers after enrollment in the first 20 subjects. Samples were tested at Days 0, 2, 6, and 28. Results demonstrated relative stability of D-dimer and fibrinogen levels in these subjects over time from enrollment.

Aim 2: MP counts have been obtained in the first 6 subjects (Fig 2 below). But since we are blinded to the clinical status of the study subjects, we cannot interpret this data for clinical significance and implications.

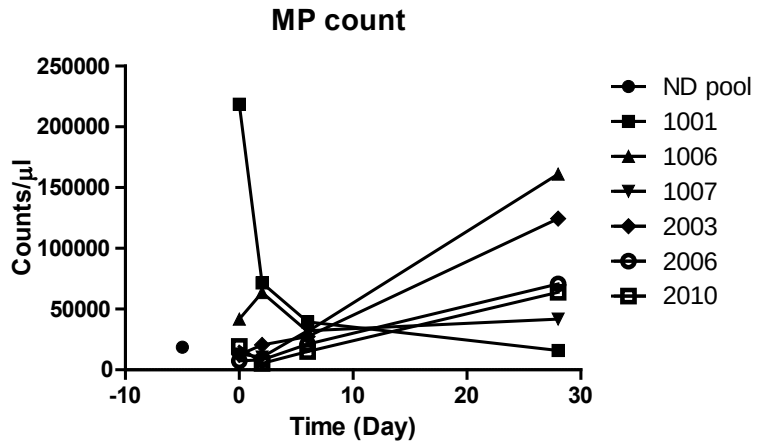

Fig. 2 Evolution of microparticle counts after enrollment in the first 6 subjects. Samples were tested at Days 0, 2, 6, and 28. Control MPs were tested using MPs pooled from 3 normal donors. Results show early elevated MPs in some subjects, with late elevations in others compared to controls.

Aim 3: The first steps in microchimerism testing have been accomplished, namely defining the transfusion recipient alleles. We have performed HLA typing on 54 patients' Day 0 samples for whom Day 0 and Day 28 (or Day 180) samples were available. These samples were typed for 12 Insertion/Deletions polymorphisms: SO1, SO3, SO4B, SO4, SO6, SO7, SO7B, SO8, SO8B, SO9, SO10 and SO11. There are 12 HLA-DRB1 low resolution types the samples were amplified for: 01, 03, 04, 07, 08, 09, 10, 11, 12, 13, 15 and 16. The next steps in measuring the presence of microchimerism will be to use real-time PCR to amplify the panel of alleles to detect minor, donor-derived populations longitudinally across each of the recipient's samples.

KEY RESEARCH ACCOMPLISHMENTS

- We continue to enroll patients and collect samples at the clinical sites in Canada
- The samples are being processed, shipped and stored.
- To date, 82 patients have been enrolled.
- We have completed coagulation testing on 90 patients' samples.
- We have completed DNA typing for Day 0 samples for 54 patients.
- We have completed microparticle testing on all samples from 6 patients.

REPORTABLE OUTCOMES

We have continued building a repository of plasma, PBMCs and whole blood samples. We have begun coagulation, micro particle and microchimerism testing.

CONCLUSION

The ABLE ancillary study has continued to enroll patients and collect samples. All sites are currently active and enrolling patients. We have begun analyzing available patient samples for

coagulation, microparticles, and microchimerism. Testing will accelerate in the coming year as we have a critical mass of samples to perform batch testing for the immunology and coagulation assays. Microchimerism testing will also continue as samples are acquired.

REFERENCES

None

APPENDICES

None